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PATENT
0508-1044

IN THE U.S. PATENT AND TRADEMARK OFFICE BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re application of	Appeal No.
Jacques Alexandre HATZFELD et al.	Conf. 5595
Application No. 09/980,484	Group 1632
Filed March 25, 2002	Examiner T. Ton

PROCESS FOR THE MULTIPLICATION OF STEM CELLS

APPEAL BRIEF

MAY IT PLEASE YOUR HONORS:

(i) **Real Party in Interest**

The real party in interest in this appeal is assignee, Centre National de la Recherche Scientifique of Paris, France.

(ii) **Related Appeals and Interferences**

None.

(iii) **Status of Claims**

Claims 1, 8-9, 11 and 33-36 are pending. The present appeal is taken from the final rejection of all of pending claims 1, 8-9, 11 and 33-36. Claims 2-7, 10, 12-32 and 37 have been canceled.

(iv) **Status of Amendments**

An Amendment After Final Rejection was filed on October 29, 2007. The Advisory Action mailed November 29, 2007 indicated that the amendment of October 29, 2007 was entered for purposes of appeal.

(v) **Summary of Claimed Subject Matter**

The present inventors have discovered an admittedly novel process for the multiplication of stem cells. The process multiplies stem cells while maintaining stem cells in a non-differentiated state (pg. 1, lines 1-5).

Claim 1 recites a method for maintaining a non-differentiated state of human stem cells, while allowing cell division of the human stem cells (pg. 1, lines 1-5), comprising administering to the human stem cells an effective amount of an inhibitor of cell development (pg. 5, lines 20-30) in sequential combination with an anti-inhibitor of cell proliferation (pg. 7, lines 1-14) in a controlled manner to maintain the non-differentiated state of stem cells, while allowing their cell division until the amplification of the cells is sufficient to obtain a pre-determined number of cells, wherein the anti-inhibitor is anti-TGFβ in an amount of 10^{-18} mg/ml to 10 mg/ml, and wherein the inhibitor is TGFβ in an amount of 0.01 pg/ml to 1 mg/ml and the human stem cells are hematopoietic stem cells (pg. 5, lines 20-30; pg. 6, lines 30-35; and pg. 7, lines 1-14).

Claim 33 recites a method for maintaining a non-differentiated state of human stem cells, while allowing cell division of the human stem cells (pg. 1, lines 1-5), comprising repeatedly administering to human stem cells in a cell concentration of about 1 to about 10^{10} cells per ml (pg. 6, lines 30-35) an effective amount of an inhibitor of cell proliferation of cell development in sequential combination with an anti-inhibitor in a controlled manner to maintain the non-differentiated state of stem cells, while allowing their cell division until the amplification of the cells is sufficient to obtain a pre-determined number of cells, wherein the anti-inhibitor is anti-TGFβ in an amount of 0.1 μg to 10 mg/ml (pg. 7, lines 1-14), and wherein the inhibitor is TGFβ in an amount of 0.01 pg/ml to 1 mg/ml and the human stem cells are hematopoietic stem cells (pg. 5, lines 20-30).

(vi) **Grounds of Rejection to be Reviewed on Appeal**

The sole issue on appeal is whether claims 1, 8, 9, 11, and 33-36 are obvious under 35 USC §103(a) in view of HATZFELD et al. (Exp. Hematology, 25(8):777 (1997) Meeting Abstract Number 174) in view of FORTUNEL et al. (J. of Cell Science, 111: 1867-1875 (1998).

(vii) **Arguments**

HATZFELD ET AL. IN VIEW OF FORTUNEL ET AL.
FAIL TO RENDER OBVIOUS CLAIMS 1, 8, 9, 11, AND 31-36

HATZFELD is an abstract of a meeting from a plenary session discussing stem cell manipulation. The abstract states that HATZFELD is studying the release of TGF-β growth inhibition on High Proliferative Potential- Quiescent primitive progenitors to understand whether the inhibitor is a central regulator of the stem cell compartment. There is no suggestion of using the of providing a process that would maintain a non-differentiated state of human stem cells, while allowing cell division of the human stem cells. The Final Rejection acknowledged that HATZFELD fails to disclose or suggest the recited amounts of anti-inhibitor or inhibitor recited in the claimed invention.

In an effort to remedy the deficiencies of HATZFELD for reference purposes, the Final Rejection cites to FORTUNEL.

FORTUNEL discloses a working model to determine whether TGF-β1 plays a role in controlling the quiescence of hematopoietic primitive cells. In doing so, FORTUNEL provides a working model of "High Proliferative Potential-Quiescent cells" to refer to primitive hematopoietic multipotent progenitors that are sensitive to the growth inhibitory effect of TGF-β1. FORTUNEL believes that the model could be used to study not only human hematopoietic quiescent progenitors but for other somatic stem cell systems (see abstract). However, there is no suggestion of

maintaining a non-differentiated state of human stem cells, while allowing cell division of said human stem cells.

Appellants believe that the proposed combination of reference fails to render obvious the claimed invention. HATZFELD primarily focuses on the effect of TGFβ and anti-TGFβ on various receptors, and more particularly pertains to the use of anti-TGFβ for rendering quiescent hematopoietic progenitors sensitive to cytokine stimulation. Contrary to the assertions of the Final Rejection and Advisory Action, HATZFELD does not disclose nor suggest how human stem cells can be multiplied in vitro while being maintained in a non-differentiated state, or the beneficial effect obtained by adding an inhibitor of cell development such as TGFβ for maintaining a "stem" state during cell divisions.

Neither does HATZFELD suggest how to use TGFβ and anti-TGFβ in a sequence combination or cyclically. Indeed, it is believed that the skilled artisan would be deterred by HATZFELD from using TGFβ or anti-TGFβ to multiply non-differentiated stem cells, since HATZFELD discusses the possibility of using "transient activation of HPPQ" as "an excellent tool to mark stem cells and follow their development", which suggests pushing the cells towards further differentiation instead of maintaining them in a non-differentiated state. Thus, appellants believe that the publication actually leads one skilled in the art away from the claimed invention.

As to the excerpt "*this possibility of rendering quiescent primitive progenitors responsive to optimal combinations of cytokines can be used to improve in vitro expansion of clinical samples*", this excerpt from HATZFELD indicates that the purpose is for a "*transient activation*" of stem cells by utilizing a number of different cytokine combinations. The purpose of expansion is not self-renewal and does not disclose or suggest administering an inhibitor and anti-inhibitor recite din the claims.

In this regard, HATZFELD does not teach a way to keep the proliferating cells in an undifferentiated state, nor the use of anti-TGFβ in a sequential manner with TGFβ to inhibit the differentiation of the cells.

It is believed that FORTUNEL fails to remedy the deficiencies of HATZFELD for reference purposed for the reasons that follow.

FORTUNEL also relates to High Proliferative Potential-Quiescent (HPP-Q) assay which can be used as a working model to study primitive quiescent haematopoietic cells. While FORTUNEL studies the effects of anti-TGf-β1 or TGf-β1 on hematopoietic stem cells, nowhere does FORTUNEL suggest administering the two components together as recited in the claimed invention. Rather, FORTUNEL focus on the effects of adminstering anti-TGf-β1 or TGf-β1 alone or with other cytokines. Thus, there is no suggestion

of administering anti-TGf-β1 and TGf-β1 together as recited in the claimed invention.

As a result, it is believe that the proposed combination of HATZFELD and FORTUNEL fail to render obvious the claimed invention.

Indeed, the disclosures of HATZFELD and FORTUNEL are at best cumulative to what is already disclosed in the present specification. Both publications discuss using a HPP-Q assay. The HPP-Q assay was developed to evaluate the differentiation potential of stem cells (e.g., the multipotentiality of adult stem cells or the pluripotentiality of human embryonic stem cells). Accordingly, those cells can then be used to study the development of the stem cells.

The HPP-Q assay is also distinguishable from the claimed invention in that the assay utilizes a very-short term proliferation of cells that grow as colonies in a semi-solid medium, which is not suitable for carrying out the multiplication of stem cells without differentiation as recited in the claimed invention.

Thus, in view of the above, applicants submit that HATZFELD and FORTUNEL fail to render obvious the claimed invention.

The Supreme Court recently addressed the issue of obviousness in *KSR International Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 167 L.Ed.2d 705 (2007). While the *KSR* Court rejected a

rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. *KSR*, 127 S.Ct. at 1731.

Moreover, the Court indicated that there is "no necessary inconsistency between the idea underlying the TSM test and the *Graham* analysis." Id. As long as the test is not applied as a "rigid and mandatory" formula, that test can provide "helpful insight" to an obviousness inquiry. Id. As neither HATZFELD nor FORTUNEL provide a reason to administer anti-TGf-β1 and TGf-β1 as recited in the claimed invention, it is believed that one skilled in the art would lack a reason to combine and modify the two publications in a manner so as to obtain the claimed invention.

As neither HATZFELD nor FORTUNEL disclose a process of stem cell production as recited in the claimed invention, applicants submit that one skilled in the art would have lacked a reason to modify the publications to obtain the claimed invention.

In view of the above, applicants respectfully request that the obviousness rejection be reversed.

Conclusion

From the foregoing discussion, it is believed that the rejection of claims 1, 8-9, 11 and 33-36 are improper and should be reversed. Such action is accordingly respectfully requested.

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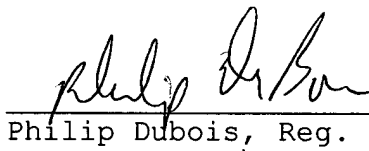
Respectfully submitted,

YOUNG & THOMPSON



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(viii) **Claims Appendix**

1. A method for maintaining a non-differentiated state of human stem cells, while allowing cell division of said human stem cells, comprising administering to said human stem cells an effective amount of an inhibitor of cell development in sequential combination with an anti-inhibitor of cell proliferation in a controlled manner to maintain the non-differentiated state of stem cells, while allowing their cell division until the amplification of the cells is sufficient to obtain a pre-determined number of cells, wherein said anti-inhibitor is anti-TGFβ in an amount of 10^{-18} mg/ml to 10 mg/ml, and wherein said inhibitor is TGFβ in an amount of 0.01 pg/ml to 1 mg/ml and said human stem cells are hematopoietic stem cells.

8. The method according to claim 1, wherein the stem cells are present in a cell concentration of about 1 to about 10^{10} cells per ml.

9. The method according to claim 1, wherein the administering of an inhibitor of cell development is performed by synthesis by the stem cells, and/or addition to the culture medium containing the stem cells.

11. The method according to claim 1, wherein the inhibitor of cell development is present in a concentration

ranging from about 10^{-10} mg/ml to 1 mg/ml.

33. A method for maintaining a non-differentiated state of human stem cells, while allowing cell division of said human stem cells, comprising repeatedly administering to human stem cells in a cell concentration of about 1 to about 10^{10} cells per ml an effective amount of an inhibitor of cell proliferation of cell development in sequential combination with an anti-inhibitor in a controlled manner to maintain the non-differentiated state of stem cells, while allowing their cell division until the amplification of the cells is sufficient to obtain a pre-determined number of cells, wherein said anti-inhibitor is anti-TGFβ in an amount of 0.1 µg to 10 mg/ml, and wherein said inhibitor is TGFβ in an amount of 0.01 pg/ml to 1 mg/ml and said human stem cells are hematopoietic stem cells.

34. The method according to claim 33, further comprising the following steps:

a) initiating a first cycle of division of said non-differentiated stem cells, by seeding non-differentiated stem cells in a resting state in an initial cell concentration, in the presence of one or more cytokines, and neutralizing the effect of an inhibitor of cell development present in the culture medium so that said cells leave their resting state by the initiation of a first cell division,

b) returning said cells to a resting state by treating said cells with an inhibitor of cell development, said inhibitor being synthesized by said stem cells or being added to the culture medium,

c) optionally washing said cells obtained in the preceding stage to remove catabolites and the inhibitor of cell development,

d) optionally diluting said cells obtained in the preceding stage to maintain an optimum cell concentration ranging from about 100 to 10^{10} cells per ml,

e) repeating the cycles of division and resting described above until the amplification factor of the cells is sufficient to obtain the number of said cells, and

f) stopping of the multiplication of non-differentiated stem cells to store them, use them or cause them to differentiate *in vitro*.

35. The method according to claim 34, wherein neutralization of the effect of the inhibitor of cell development, present in the culture medium is effected by

- addition to the culture medium, in a suitable amount, of an anti-inhibitor of cell proliferation, and

- withdrawal from the culture medium of the inhibitor of cell development.

36. The method according to claim 34, wherein the duration of a single resting state ranges from 1 hour to 3 years and in that the duration of a single division cycle ranges from about 6 hours to 3 years.

(ix) **Evidence Appendix**

None.

(x) **Related Proceedings Appendix**

None.